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Award Number: **W81XWH-11-2-0084**

TITLE: Optimization of Lyophilized Plasma for Use in Combat Casualties

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REPORT DATE: January 2013

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	**2. REPORT TYPE**	**3. DATES COVERED**
21 January 2013	Annual	29-December 2011 - 28 December 2012

4. TITLE AND SUBTITLE
Optimization of Lyophilized Plasma for Use in Combat Casualties

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-2-0084

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Oregon Health & Science University
Portland, Oregon 97239

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Purpose: Hemostatic resuscitation is superior to crystalloid resuscitation in restoring blood volume, correcting coagulopathy, minimizing dysfunctional inflammation, and acidosis. Resuscitation with 'full volume' lyophilized plasma (LP) reduces blood loss, corrects coagulopathy, and decreases inflammation in a swine polytrauma and hemorrhagic shock model. This study compared the efficacy of 'full volume' LP to 'low volume' hypertonic LP. **Scope:** Prior to in vivo testing, in vivo analysis of the plasma was analyzed for electrolyte content, osmolarity and coagulation factor activity. Twenty swine were anesthetized and subjected to a validated model of polytrauma and hemorrhagic shock. They were randomized with lyophilized plasma (LP) reconstituted to either 100% (100%LP) or half (50%LP) the original plasma volume. Physiologic data were monitored. Blood loss and hematocrit levels were measured. Coagulation status was evaluated utilizing thrombelastography (TEG). Serum and tissue were collected to assess inflammatory markers. **Major Findings:** 50%LP had higher electrolyte concentrations, osmolarity, and increased coagulation factor activity levels by volume compared to 100%LP. Blood loss, hematocrit, mean arterial pressure and heart rate were not significantly different between groups at any time point. In addition, there were no differences denoted in TEG values. With these finding it is safe to say 50%LP is equally effective as 100%LP. The smaller volume of fluid necessary to reconstitute the 50%LP makes it logistically superior to 100%LP and may reduce adverse effects of large fluid volume resuscitation.

15. SUBJECT TERMS
Uncontrolled hemorrhage, resuscitation, animal model, coagulation, swine, lyophilized plasma

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			**19b. TELEPHONE NUMBER** *(include area code)*
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INTRODUCTION:

Trauma is the leading cause of deaths among civilians between the ages of 1 and 44[1]. Each year, trauma is responsible for 37 million emergency department visits and 2.6 million hospitalizations. Uncontrolled hemorrhage accounts for approximately 40% of these deaths [2, 3]. The traditional method of treating uncontrolled traumatic hemorrhage is prompt and aggressive fluid resuscitation with crystalloid to restore circulating volume and systolic blood pressure [4, 5]. This approach to traumatic resuscitation is reflected by the American College of Surgeons Committee on Trauma's current recommendation to resuscitate all trauma patients with two or more liters of lactated Ringer's (LR) or normal saline (NS) [6]. Along with the hypovolemic shock associated with the acute loss of intravascular volume in hemorrhaging trauma, concurrent severe tissue injury also initiates massive activation of the coagulation system, resulting in a consumptive coagulopathy and hypocoagulable state [7-9]. Additionally, there has been an abundance of literature demonstrating multiple detrimental effects resulting from large volume crystalloid resuscitation including worsening coagulopathy, acidosis, hypothermia, and increased inflammation [3, 10-12]. The net effect is not only the initiation but also the amplification of the acute coagulopathy of trauma (ACT).

Coagulopathy in trauma is directly associated with poor outcomes [3]. Critical in reversing or minimizing ACT is prompt transfusion of component blood products to replace blood volume, oxygen carrying capacity, and the components of coagulation [13]. Studies performed by multiple groups have shown that aggressive and early use (ideally within the first 6 hours of injury) of component blood therapy is associated with lower mortality in massively transfused trauma patients. Though the precise ideal ratio of fresh frozen plasma (FFP) to red blood cells (RBC) is not yet defined, these studies suggest that ratios approaching 1:1 of FFP:RBC significantly improves overall survival [14-17].

The logistical difficulties associated with the collection, storage, and thawing of FFP for use makes meeting these high ratio requirements difficult in many centers. Additionally, these cumbersome storage requirements make FFP unavailable for civilian first responders and far-forward combat personnel. Lyophilized plasma (LP) can be stored at room temperature, easily transported, and quickly reconstituted for use. Prior work has shown that LP reconstituted to 100% of the original plasma volume retains on average 86% of the pre-lyophilization coagulation factor activity [18]. Additionally, the same study demonstrated that fully reconstituted LP was as effective as FFP in terms of hemodynamic parameters and blood loss when used for resuscitation in a swine model of polytrauma and severe hemorrhage [18].

We hypothesized that by minimizing the volume needed to reconstitute LP, we can create a low volume hemostatic resuscitation fluid without loss of hemostatic efficacy. This low volume LP would make it superior to FFP with respect to logistics, hemodynamic changes, coagulopathy, and blood loss in an established swine model of polytrauma and hemorrhage.

BODY:

Materials and Methods

<u>Specific Aim 1 Materials</u> – To determine the minimum amount of fluid necessary to successfully reconstitute lyophilized plasma without reducing its efficacy in a swine multiple injury model of hemorrhagic shock.

This model was developed at Oregon Health & Science University (OHSU), and approved by the Institutional Animal Care and Use Committee.

Female Yorkshire Crossbred swine underwent the following polytrauma protocol to assess the efficacy that lyophilized plasma reconstituted with a smaller volume will result in similar or better results than fully reconstituted lyophilized plasma.

<u>Specific Aim 1 Methods</u> – Efficacy of Hypertonic Lyophilized Plasma

Blood Collection for Plasma Preparation

All experimental procedures were done in accordance with the guidelines of the Institutional Animal Care and Use Committee at Oregon Health & Science University. Blood products used in this study were obtained from juvenile Female Yorkshire crossbred swine. Using sterile precautions, a cervical cut down was performed and the carotid artery was cannulated with an 8F introducer (Argon Medical Devices, Athens, Texas). Animals were exsanguinated and blood was collected into citrated blood donation bags (Teruflex; Terumo Medical Corp, Tokyo, Japan). Whole blood was centrifuged at 5000g for 9 minutes at 4°C. The plasma was then removed using a plasma extractor (Baxter Healthcare, Deerfield, Illinois). Plasma was stored at -20°C for transport to a laboratory (HemCon Medical Technologies Inc, Portland, Oregon) for lyophilization. Pooled sterile LP was returned to us and stored at room temperature for up to 2 months. To ascertain the minimal volume sufficient for reconstitution of LP, reconstitution was done using decreasing volumes of sterile water with ascorbic acid as buffer. We decreased the volume in stepwise fashion using 10% volume increments. Immediately prior to use, LP was reconstituted at the specified volume with sterile water containing ascorbic acid for pH adjustment.

Plasma Clotting Factor Level Measurements:

Samples of the plasma were analyzed for fibrinogen, coagulation factors II, V, VII, VIII, IX, X, XI, XII, and XIII using a coagulation system machine (BCS; Dade Behring Inc, Marburg, Germany) at the time of plasma collection and following reconstitution of each study fluid. Blood from each study animal was also collected for coagulation factor analysis at all time points during the study.

Animal Model:

20 juvenile, female Yorkshire crossbred swine were subjected to a well-validated swine model of severe injury and hemorrhagic shock (Figure 1). Animals were delivered 7 to 10 days prior to the experiment in order to minimize the stress of transport and subsequent potential changes in sympathetic output or inflammatory mediators. Animals were fasted for 16 hours the day before surgery. Water was available ad libitum. A single vendor was utilized to eliminate potential differences in animal strain.

Anesthesia

On the day of the experiment animals were given an induction agent consisting of 8 mg/kg Telazol® (tiletamine hydrochloride 50 mg/ml, zolazepam hydrochloride 50 mg/ml, Fort Dodge Animal Health, Fort Dodge, Iowa) given intramuscularly. Animals were placed in the supine position. Orotracheal intubation was performed and a 7.5mm internal diameter cuffed endotracheal tube was placed. The endotracheal tube was connected to the anesthesia machine with 1-3% isoflurane for anesthetic maintenance in 50% oxygen. Tidal volume was fixed at 10 ml/kg with a rate of 10 breaths per minute. An esophageal stethoscope, gastric tube and thermometer were inserted. An EKG monitor was secured and continuous monitoring started. Throughout the study, anesthesia was maintained to the clinical endpoints of reflexes and muscle relaxation as is done in humans.

Monitoring, access and pre-experiment procedures

After swine were anesthetized a left cervical cutdown was performed and polyethylene catheters were inserted respectively into the left common carotid and left external jugular vein. The arterial line was utilized for the controlled hemorrhage and blood sampling throughout the experiment while the venous line was used for administration of bolus resuscitation fluids and TXA. Finally, a proximal femoral cutdown was performed and the artery was cannulated for continuous blood pressure monitoring. Mean arterial pressure (MAP) was continuously recorded and averaged every 10 seconds with a blood pressure analyzer and digital data collection system (DigiMed, Louisville, KY). Baseline labs were collected and included electrolytes, lactate, spun hematocrit (Hct), activated clotting time (ACT), platelets (Plt), prothrombin time (PT), partial thromboplastin time (PTT), and arterial blood gas (ABG) and additional coagulation factors. In addition, a baseline thrombelastogram (TEG, Haemoscope Corporation, Niles, IL) was performed. A celiotomy was then performed, at which time a suprapubic bladder catheter was placed to monitor urine output.

Injury Phase

After needle localization, a captive bolt gun was used to fracture the femur and create a soft tissue injury at the midshaft of the left femur. A controlled hemorrhage was then initiated to remove 60% of the blood volume based on a published, standard equation relating blood volume to body weight for domestic swine. During hemorrhage if the mean arterial blood pressure (MAP) fell below 25mm/Hg, normal saline (NS) was infused at a rate of 165 ml/min to keep the MAP>25 mm/Hg. The animal was also cooled to 33 +/-0.4^{o}C using cooled intraperitoneal lavage with crystalloid as needed (most of the animals developed a degree of hypothermia spontaneously due to shock and infusion of IV fluids). These procedures were followed by a 30-minute shock period, representing time in the field prior to medical intervention.

Prehospital care/transport phase

After the 30-minute shock period, electrolytes, spun hematocrit, ACT, PT, PTT, platelets, ABG, factors and TEG were again recorded. After coagulation studies and lab collection, the hemorrhage volume was replaced with a 3:1 ratio of NS infused at a rate of 165 ml/min, minus any given during the controlled hemorrhage to induce acidosis and coagulopathy. This reflects current civilian pre-hospital resuscitative practices.

Operative phase

Following NS resuscitation, a 15-minute stabilization period was observed; during which a baseline MAP was recorded and pre-weighed laparotomy sponges were placed in both paracolic gutters and in the pelvis for blood collection. Labs and coagulation studies were again collected, and a previously described grade V liver injury was created at the confluence of the right and middle hepatic veins using a specialized clamp. The liver injury was designed to provide a second stressor after initial injury and also to create a standardized injury that had the potential to re-bleed, both of which simulate a laparotomy after trauma in a patient with solid organ injury.

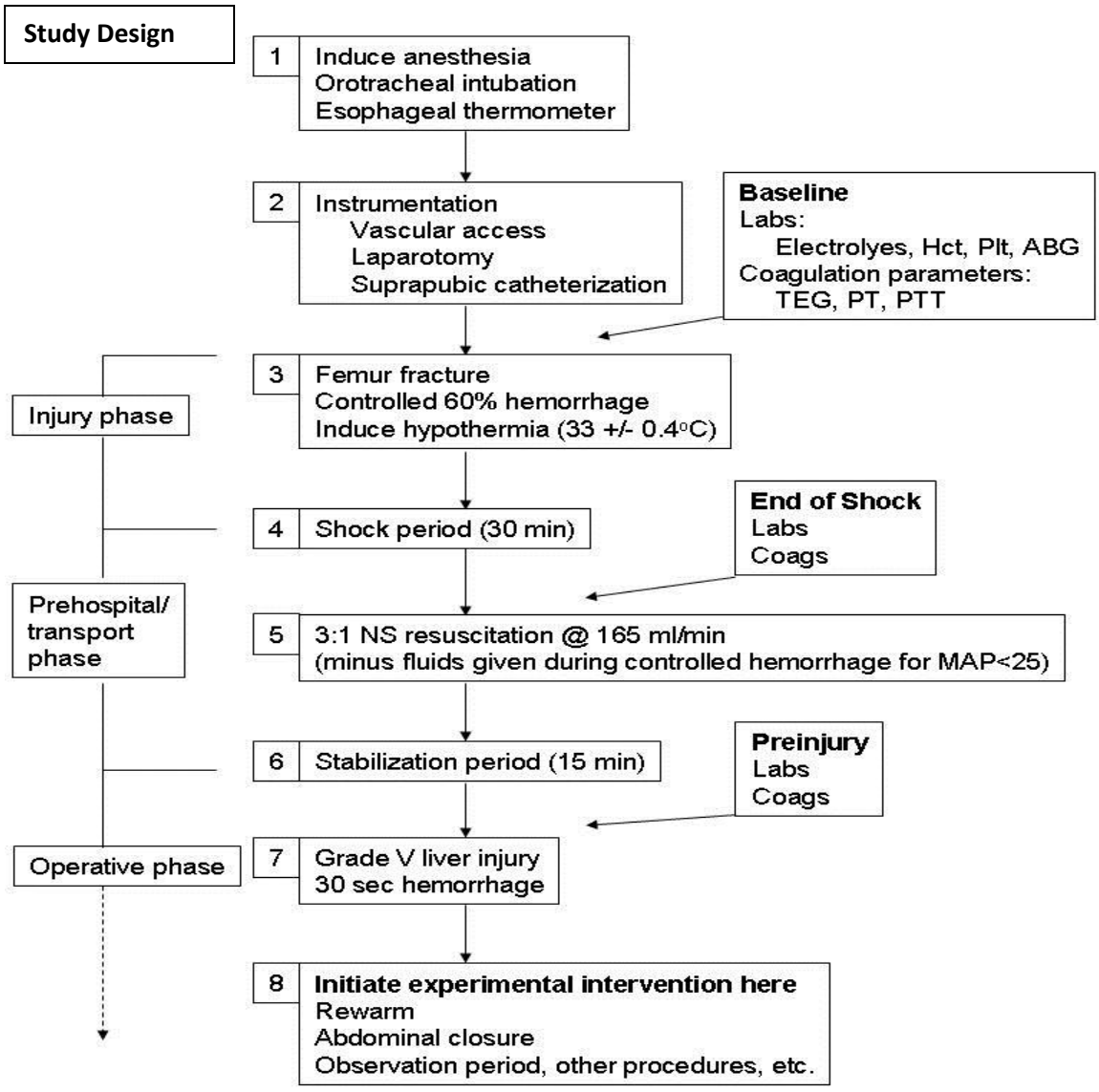
Thirty seconds of hemorrhage were then followed by evacuation of blood from the abdomen. Following the uncontrolled hemorrhage period, the liver was packed tightly with laparotomy sponges. Swine were randomized to receive either LP reconstituted to 50% (50%LP, n=10) or 100% (100%LP, n=10) of the original plasma volume. Study fluid resuscitation was initiated at the time of liver packing. The animal was also re-warmed to 37°C and the abdomen closed with towel clips.

Follow-up

Animals were monitored for 4 hours post injury or to death. Labs were collected at 1, 2, 3 and 4 hours. If the MAP fell below 15 mmHg it was denoted as death and the time of death was recorded. Animals surviving 4 hours were euthanized with Euthasol.

Lung tissue was collected at the end of 4 hours or at declaration of death for rtPCR analysis. Tissue was stored in RNA later and a 10% buffered formalin solution. A necropsy was performed and the liver injury graded using the American Association for the Surgery of Trauma (AAST) liver injury grading system to ensure adequacy and similarity of injuries between groups.

Heart (HR) rate and blood pressure (MAP) were continuously recorded throughout the study. Blood loss following liver injury was carefully recorded with the use of pre-weighed laparotomy sponges and pre-weighed suction canisters.



Study Variables

Physiologic variables included survival, MAP, blood loss from the controlled hemorrhage, and blood loss due to the liver injury. Laboratory values include Hct, lactate, Plt, ABG, and electrolytes. Coagulation parameters include the PT, PTT, ACT, and Factors II, V, VII, VIII, IX, X, XI, XII.

Statistical Analysis

Variables were assessed for normal distribution. Normally distributed data were reported as means with standard deviations. Comparisons between groups at various time points were analyzed by independent t-tests when the data were normally distributed. Paired-samples t-tests were used to compare same-group samples across various time points. Significance was denoted at $p < 0.05$. Data were analyzed utilizing SPSS statistical software, version 19.0 (IBM Corp. Released 2010. Armonk, NY).

Results

In vitro

In vitro analysis of LP was successfully reconstituted using 30% of the original plasma volume. However, when intravenously administering LP reconstituted to 30% and 40% of the original plasma volumes to animals during model development, the fluids were not well tolerated. These animals died prior to or shortly after completion of the LP fluid infusions. The 50%LP solution was well tolerated in all animals.

The 50%LP solution had significantly higher concentrations of electrolytes and albumin (Table 1). Additionally, 50%LP was hyperosmolar with significantly higher osmolarity compared to the 100%LP solution. The pH of the two study fluids following reconstitution using sterile water with ascorbic acid as buffer was not different (Table 1).

Regarding coagulation factor activity, there was no significant difference between pre-lyophilized plasma (FFP) and 100%LP (Figure 1). We found significantly increased coagulation factor activity (Fibrinogen, II, V, VII, VIII, IX, X, XI, and XII) per unit volume in the 50%LP fluid compared to FFP and 100%LP (all $p < 0.03$). TEG parameters were not different at any time point between the fluid study groups (R time, K, α – angle, or MA, $p > 0.17$).

In vivo

All 20 swine randomized to receive either 50%LP (n=10) or 100%LP (n=10) study fluid survived the study period. At baseline, animals were similar between study fluid groups (Table 2). Serum lactate increased in both study groups following femur fracture and controlled hemorrhage (Figure 2). There was no significant difference in changes in serum lactate between study groups throughout the study at any time point (Figure 2). There was no statistically significant difference in blood loss at any time point following liver injury and total blood loss at the end of the study period between the study groups (Figure 3). There was also no significant difference in hemodynamic parameters (HR and MAP) between study groups (Figure 4). No difference in hematocrit (Hct) was found between study groups at any time point (Figure 4). Analysis of the coagulation and inflammatory markers are currently ongoing for the in vivo aspect of the project.

KEY RESEARCH ACCOMPLISHMENTS – Specific Aim 1

1. Less than 50% reconstitution not well tolerated but soluble
2. 50%LP displayed significantly greater activity of all coagulation factors
3. No statistically significant differences between the groups at baseline
4. No statistically hemodynamic or laboratory difference between animals post infusion

REPORTABLE OUTCOMES – Specific Aim 1

1. 39th Annual Critical Care Symposium – Oregon Chapter of Society of Critical Care Medicine, November 12 – 13, 2012 in Vancouver, WA. . "Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma".
   Winner of Best Trainee Competition
2. Eastern Association for the Surgery of Trauma (EAST) 26th Annual Scientific Assembly, November 15 – 19, 2012 in Scottsdale, AZ. "Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma".
   Winner of Raymond H. Alexander, MD Resident Paper Competition

3. Oregon / Washington American College of Surgeons Committee on Trauma, Region X Conference, December 1, 2012 in Centralia, WA. "Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma".
   Winner of Best Basic Science Paper
4. American College of Surgeons – Oregon / Washington Regional Conference, June 16, 2012 in Sunriver, OR. "Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma".
   Winner of Baker-Mosely Award for Excellence in Resident Laboratory Research
5. Oregon Health & Science University Research Week May 9, 2012 in Portland, OR. "Hypertonic reconstituted lyophilized plasma is an effective low volume hemostatic resuscitation fluid for trauma".

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	50%LP (n=8)	**100%LP (n=8)**	***p***
Na (mmol/L)	297 ± 48	171 ± 22	< 0.05
K (mmol/L)	9.2 ± 3.1	4.7 ± 1.3	0.002
Cl (mmol/L)	139 ± 30	80 ± 14	< 0.05
Ca (mmol/L)	11.0 ± 2.5	6.5 ± 0.9	< 0.05
Alb (mmol/L)	2.0 ± 0.3	1.0 ± 0.2	< 0.05
Osmolarity (osmol/L)	621 ± 118	329 ± 44	< 0.05
pH	7.11 ± 0.11	7.18 ± 0.08	0.18

Table 1. Study fluid analysis (50%LP and 100%LP). All values expressed as group mean ± SD. (Na: sodium, K: potassium, Cl: chloride, Ca: calcium, Alb; albumin)

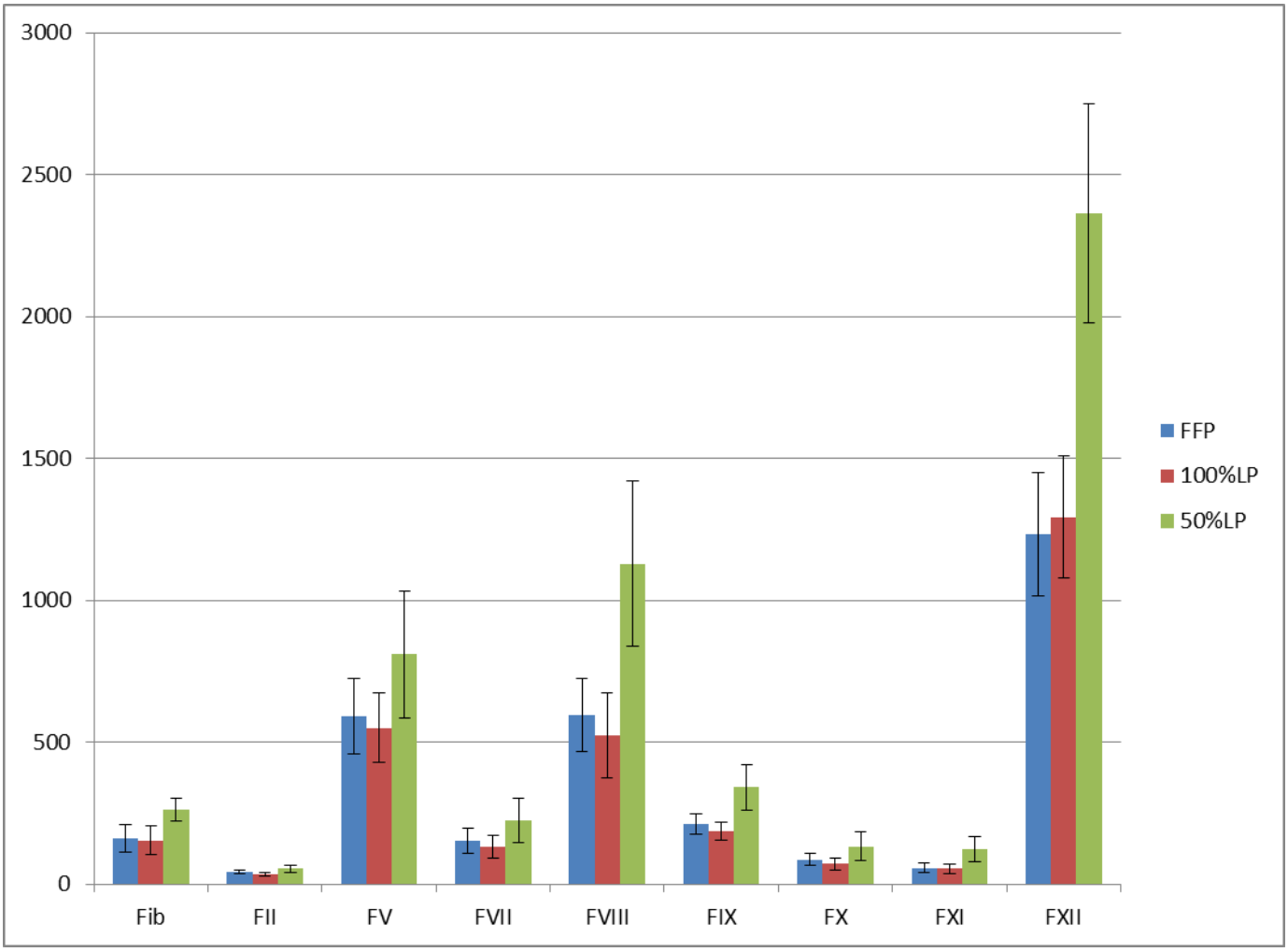

Figure 1. 50%LP has significantly greater activity of all coagulation factors compared to FFP and 100%LP, all $p < 0.03$. All values expressed as mean ± SD. (Fib: fibrinogen, FII: Factor II, FV: Factor V, FVII: Factor VII, FVIII: Factor VIII, FIX: Factor IX, FX: Factor X, FXI: Factor XI, FXII: Factor XII).

	50%LP (n=10)	**100%LP (n=10)**	***p***
Weight (kg)	32.8 ± 1.3	32.3 ± 2.0	NS
Hct (%)	28.7 ± 1.1	29.2 ± 2.1	NS
Lactate (mmol/L)	1.4 ± 0.3	1.5 ± 0.5	NS
Base excess (mmol/L)	12.9 ± 2.2	11.8 ± 1.3	NS

Table 2. Baseline characteristics of animals in each study fluid group (50%LP and 100%LP). All values expressed as mean ± SD, NS: No significant difference, all $p > 0.05$; Hct: hematocrit.

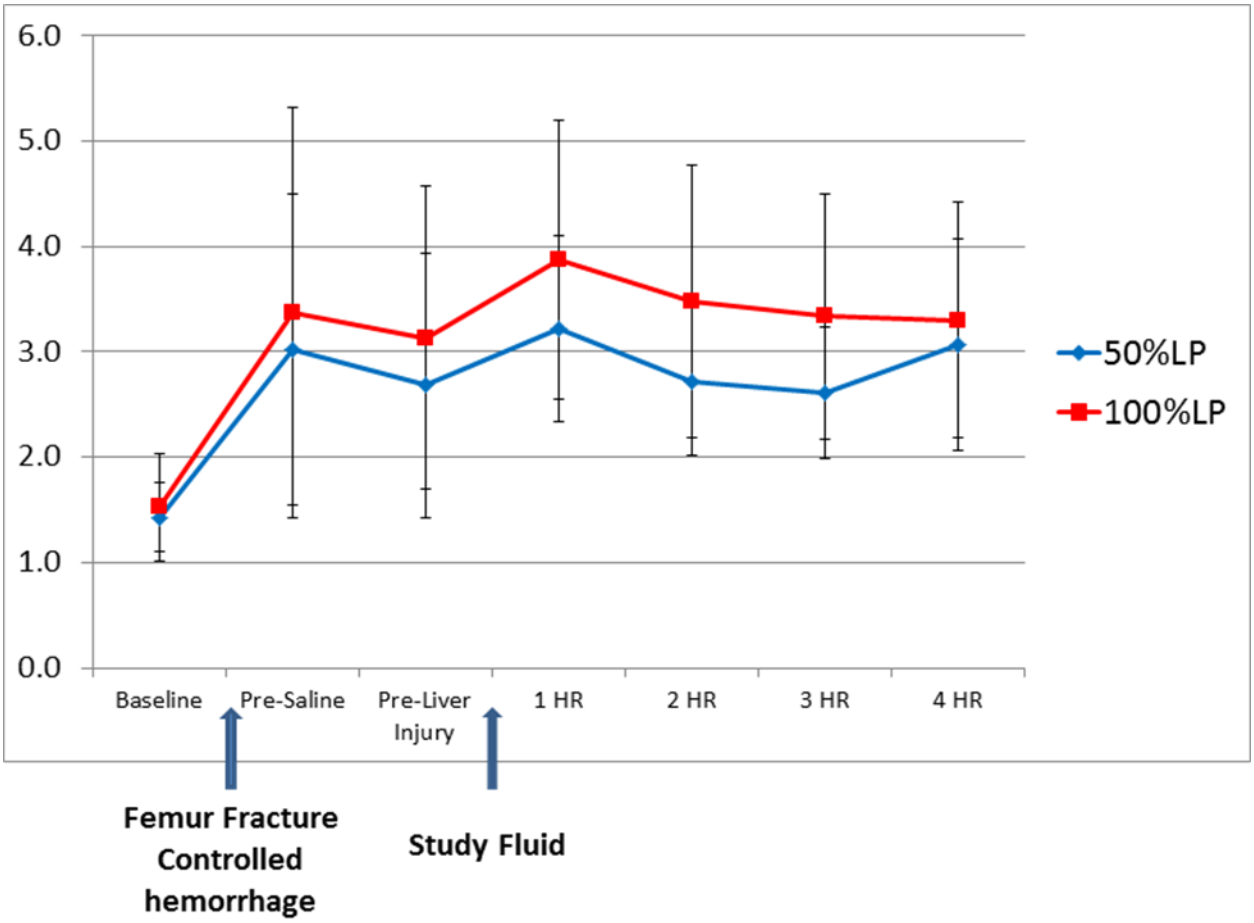

Figure 2. Serum lactate. Data presented as group mean ± SD, $p > 0.05$ at each time point.

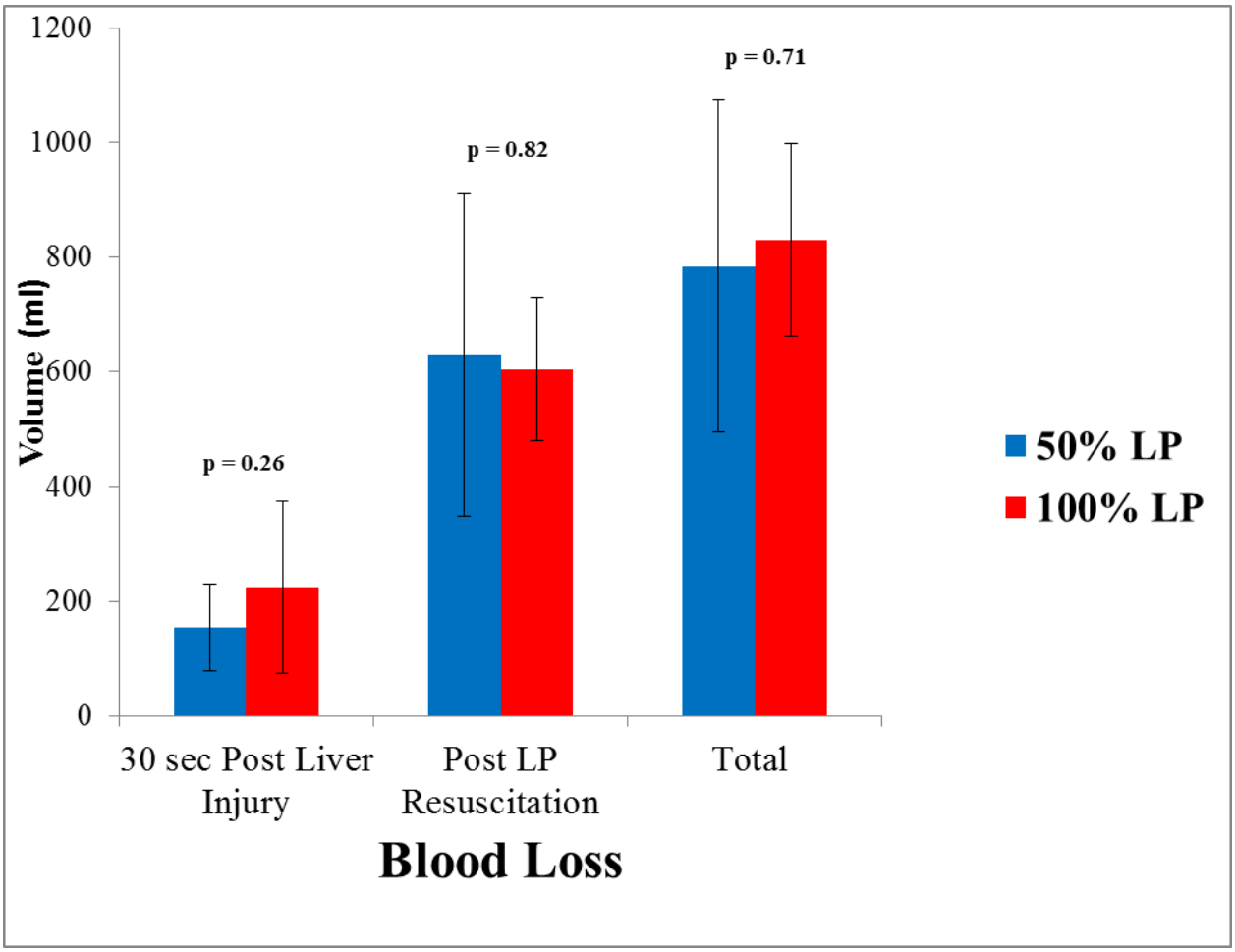

Figure 3. Blood Loss. There was no significant difference in blood loss between study fluid groups (50%LP and 100%LP) for all time points (all $p > 0.05$). All values expressed as mean ± SD.

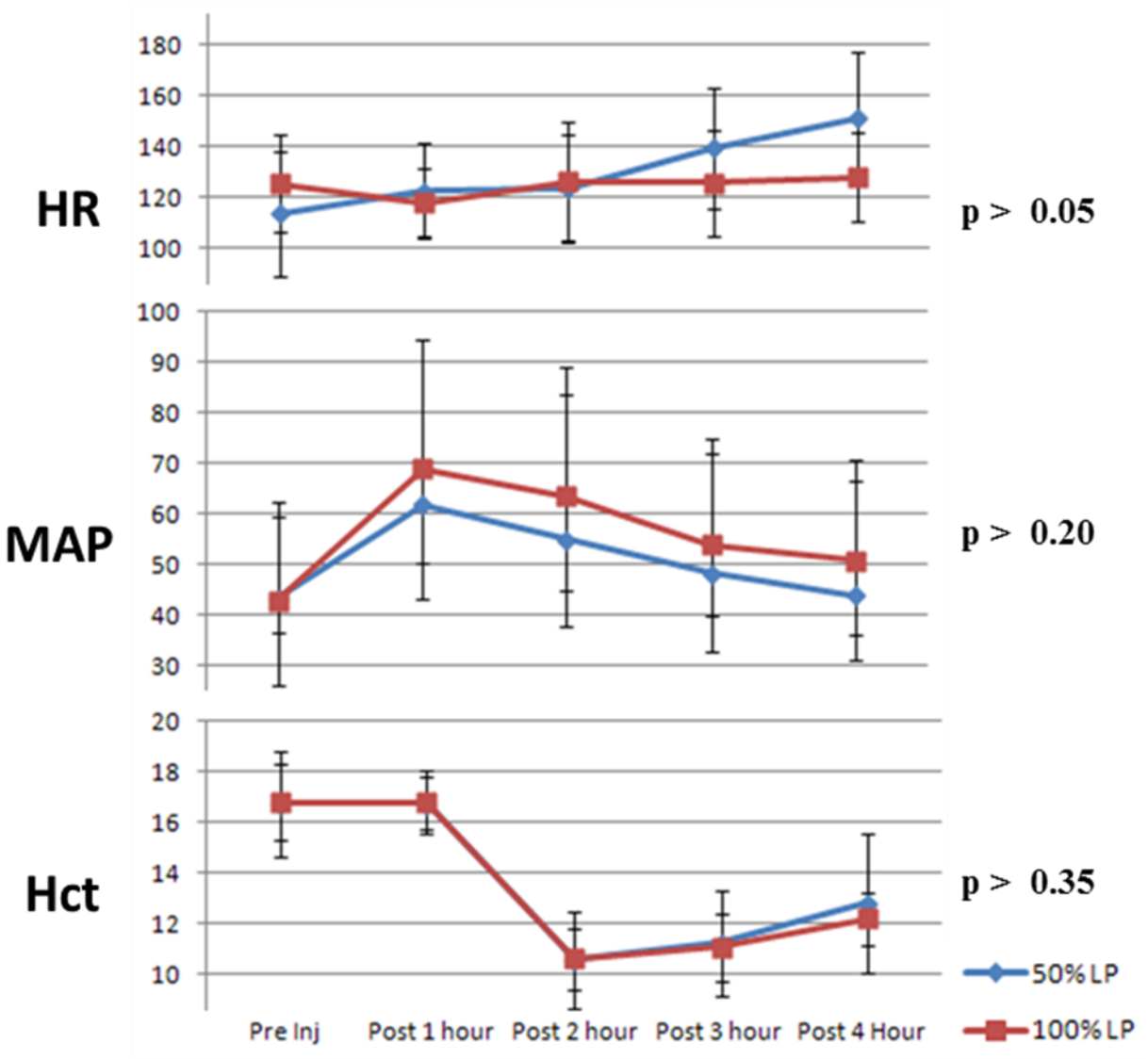

Figure 4. Hemodynamic parameters and Hematocrit (Hct). There was no significant difference in heart rate (HR), mean arterial pressure (MAP), and Hct between study fluid groups (50%LP and 100%LP). All values expressed as mean ± SD, all $p > 0.05$.